Culture and geographic variation in orangutan behavior

Krützen, M ; Willems, E P ; van Schaik, C P

Abstract: Although geographic variation in an organism's traits is often seen as a consequence of selection on locally adaptive genotypes accompanied by canalized development [1], developmental plasticity may also play a role [2, 3], especially in behavior [4]. Behavioral plasticity includes both individual learning and social learning of local innovations ("culture"). Cultural plasticity is the undisputed and dominant explanation for geographic variation in human behavior. It has recently also been suggested to hold for various primates and birds [5], but this proposition has been met with widespread skepticism [6-8]. Here, we analyze parallel long-term studies documenting extensive geographic variation in behavioral ecology, social organization, and putative culture of orangutans [9] (genus Pongo). We show that genetic differences among orangutan populations explain only very little of the geographic variation in behavior, whereas environmental differences explain much more, highlighting the importance of developmental plasticity. Moreover, variation in putative cultural variants is explained by neither genetic nor environmental differences, corroborating the cultural interpretation. Thus, individual and cultural plasticity provide a plausible pathway toward local adaptation in long-lived organisms such as great apes and formed the evolutionary foundation upon which human culture was built.

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Culture and geographic variation in orang-utan behaviour

Michael Krützen[1,*], Erik P. Willems[1], and Carel P. van Schaik

Anthropological Institute and Museum, University of Zurich, Zurich, Switzerland

[1]Both authors contributed equally to this work.

[*]Correspondence: michael.kruetzen@aim.uzh.ch (M.K.)

Running title: Plasticity and culture in orang-utans

Highlights

- Orang-utans show dramatic geographic variation in behaviour
- As in humans, plasticity plays a major role in explaining this variation
- Environment drives variation in behavioural ecology and social organisation
- Cultural processes drive variation in behaviours previously claimed to be cultural

Summary

Although geographic variation in an organism's traits is often seen as a consequence of selection on locally adaptive genotypes accompanied by canalised development [1], developmental plasticity may also play a role [2, 3], especially in behaviour [4]. Behavioural plasticity includes both individual learning, and social learning of local innovations ("culture"). Cultural plasticity is the undisputed and dominant explanation for geographic variation in human behaviour. It has recently also been suggested to hold for various primates and birds [5], but this proposition has been met with widespread scepticism [6-8]. Here, we analyse parallel long-term studies documenting extensive geographic variation in behavioural ecology, social organisation, and putative culture of orang-utans [9] (genus: *Pongo*). We show that genetic differences amongst orang-utan populations explain only very little of the geographic variation in behaviour, whereas environmental differences explain much more, highlighting the importance of developmental plasticity. Moreover, variation in putative cultural variants is explained by neither genetic nor environmental differences, corroborating the cultural interpretation. Thus, individual and cultural plasticity provide a plausible pathway towards local adaptation in long-lived organisms such as great apes, and formed the evolutionary foundation upon which human culture was built.

Results

In this study, we use the predictions of a cultural plasticity model that, if confirmed, allow us to reject other developmental causes of geographic variation in behaviour of orang-utans (*Pongo* spp.), such as canalised development under strong genetic control or individual plasticity. We apply this approach to geographic variation in behavioural ecology (activity budgets, diet and ranging), social organisation (local density, associations, and socio-sexual variables) and putative cultural behaviours, observed amongst wild populations of orang-utan in both Sumatra (*P. abelii*) and Borneo (*P. pygmaeus*), which have been subject to long-term field studies (Figure 1).

We found that orang-utan populations are genetically highly differentiated from each other. For all DNA markers used in this study, only a very small fraction of the total variance was explained by variation within populations, and differentiation measures between most pairs of populations were significant (Table 1). Thus, there is sufficient genetic variation amongst populations and islands potentially to explain geographic variation in behaviour.

Matrix permutation tests revealed several significant bivariate correlations between differences in behavioural ecology and genetic and environmental dissimilarities amongst 11 populations (Table 2). However, subsequent analyses aimed at partitioning the total observed variance into uniquely genetic and uniquely environmental components, revealed that a non-significant 4 % of the total variance in orang-utan behavioural ecology was accounted for by genetic differences between sites, whereas more than 25 % could be attributed to environmental differences (Table 2, Figure 2). Therefore, geographic variation in orang-utan behavioural ecology appears to be much better explained by local adaptation through developmental plasticity than through genetic canalization.

The documented geographic variation in social organisation amongst 7 orang-utan populations also showed several significant bivariate correlations with both genetic and environmental dissimilarities (Table 2). Subsequent estimates of the unique proportions of the total variance explained, showed that genetic dissimilarities consistently accounted for less than 7 % of variation, regardless of which genetic marker system was used, whereas environmental factors again explained more than 25 % (Table 2, Figure 2). Therefore, geographic variation in orang-utan social organisation also appears to result mainly from local adaptation through developmental plasticity rather than through genetic canalization.

Finally, geographic variation in behaviour patterns previously suggested to be cultural [10] showed non-significant bivariate correlations with both genetic and environmental dissimilarities amongst 9 populations (Table 2). Partial Mantel tests indicated that both genetic and environmental differences each accounted for a minor and non-significant proportion of the total variance observed (Table 2, Figure 2). These 10 putative cultural variants had been selected because they were not subject to environmental influences and were amongst the most conspicuous and frequent ones. However, the same was found when all 24 putative cultural elements were considered: geographic variation was not significantly associated with either explanatory variable, although environmental dissimilarities approached significance (Table S1). Therefore, neither genetic canalization nor individual plasticity can account for geographic variation in putative orang-utan culture.

We repeated all analyses using Spearman rank correlation matrix permutation tests for both genetic marker systems to control for undue influences of potential outliers and potential ceiling effects in our genetic dissimilarity measures. They confirmed all previous conclusions (Table S2).

Discussion

Virtually all species show some geographic variation in their phenotypes, from morphology and physiology to behaviour and life history [2-4]. This geographic variation is often thought to reflect differential local adaptation through the action of natural selection [11]. Perhaps because of the success of experimental approaches, typically focusing on invertebrates and fish [1], genetic variation accompanied by canalised development is usually presented as the *de facto* null model to explain geographic variation in a trait [7].

A locally adaptive phenotype might also be attained through an additional pathway, namely developmental plasticity, provided that this is not too costly [3]. This pathway is especially likely if extensive gene flow or insufficient time since separation prevents local adaptations from becoming genetically fixed [12]. However, for behavioural traits it is a plausible mechanism under all conditions, because behavioural plasticity includes learning. Indeed, the ubiquity of learning, especially in birds and mammals [4, 13], suggests that individual plasticity is a common mechanism to adjust behaviourally to local conditions.

An additional form of behavioural plasticity is the acquisition of skills or information through social learning: cultural plasticity. Social learning ranges from learning due to proximity or attraction to the same stimuli or specific locations, to learning by directly copying goals or actions [14]. Social learning provides the standard explanation for geographic variation in human behaviour, *i.e.* culture [15], yet similar propositions for non-human animals [5] remain controversial [6-8, 16].

Our analyses demonstrate that developmental plasticity plays a major role in bringing about geographic variation in orang-utan behaviour. If genetic differences had been responsible, we should have found co-variation between genetic and behavioural variation, because populations and especially islands (Sumatra vs. Borneo) were genetically highly differentiated. Nonetheless, genetic dissimilarities explained at most 7 % of the behavioural variation. In contrast, environmental variation explained more than 25 % of the variation in behavioural ecology and social organisation, supporting a major influence of developmental plasticity.

Previous cultural interpretations of geographic variation in ape behaviour have been criticized for not having incorporated the effect of environmental differences between sites [7]. Here, however, we first demonstrated that the environmental differences we measured are ecologically meaningful since they explain variation in behavioural ecology and social

organisation. Yet, they could not explain the variation in the putative cultural behaviours. Moreover, our reduced culture data set contains only those putative cultural elements that are unlikely to be linked to environmental factors. Because variation in putative cultural elements was correlated with neither genetic nor environmental variation, this particular category of geographic variation in behaviour must have come about through local innovations, spread and maintained by social learning [10, 17].

Our findings are also supported by multiple other sources of information. First, in our dataset, the contrast in social organisation was the only significant predictor of dissimilarities in conspicuous and frequent putative cultural behaviours (Table S3), which is consistent with site-specific sociability being a good predictor of the local repertoire size of putative cultural variants [10]. Second, wild immature orang-utans show selective visual attention to exactly those behaviours thought to be most difficult to acquire independently [18], nearby populations exhibit differences in diet composition and call repertoires consistent with innovation and social learning [19], and orang-utans are proficient social learners in captivity [20]. Finally, similar work on other species, especially chimpanzees [21], supports this conclusion. Thus, this study provides the strongest support to date in the ever-growing chain of evidence substantiating a cultural interpretation of geographic variation in certain elements of non-human primate behaviour [10, 17].

Although historically it has been good scientific practice to assume canalised development as the null model, we might now have to question its adequacy for long-lived animals that rely on extensive external inputs, including social ones, during development. First, long-lived animals are likely to be confronted with variation over time in environmental conditions, and being usually large-bodied also tend to roam so widely that they may encounter many different conditions. Second, these animals may not have the demographic potential to respond rapidly to selection for local adaptation, forcing them to rely more on plasticity to maintain locally adaptive phenotypes [22]. The indications for extensive social learning and cultural variation in other long-lived organisms such as dolphins [23] and whales [24], elephants [25], monkeys [26], and some birds [27] support the idea that cultural plasticity is an important pathway to local adaptation. The fact that culture is found in great apes, moreover, gives us a much better basis for developing a theoretical framework for cultural evolution, within which to address the question of the elaboration of this ability in humans [15].

Our results are entirely consistent with the cultural interpretation, by demonstrating that the proportion of geographic variation in putative cultural behaviours explained by genetic or environmental differences amongst populations is very low, but also highlighting the importance of phenotypic plasticity, of which culture is just one aspect, in long-lived animals more generally.

Experimental procedure

The cultural plasticity model

In the cultural plasticity model, plasticity (individual or cultural) is implicated if there is no correlation between genetic and behavioural variation across populations. Note that this does not mean that the behaviour itself has no genetic basis, but merely that geographic variation in its manifestation is primarily due to developmental plasticity. Because the expression of virtually all behavioural traits is caused by polygenic loci, identification of the genes potentially responsible for the geographic variation in complex behaviours is virtually impossible [28]. Therefore, the only feasible approach in wild animal populations is to use neutral genetic markers, followed by estimating the extent of genetic divergence as an index for the differences between populations in the genes causally involved in the behaviours, as done previously [29].

The use of this measure can be criticised if local selection subsequent to divergence of two populations has favoured differences among particular coding genes, which therefore became disassociated from the overall genetic dissimilarity across sites. However, selection on the polygenic traits most likely responsible for behavioural variation will be attenuated over multiple loci, so that each locus behaves as if it evolved nearly neutrally [30]. Moreover, simulations showed that genetic differentiation measures calculated from quantitative trait loci are almost identical to those derived from neutral markers, regardless of the selective regime imposed on the selective trait [31]. This fact justifies the use of overall genetic similarity measures even in the potential presence of selection on behaviour patterns.

Provided genetic and behavioural variation are uncorrelated, the plasticity interpretation is confirmed if environmental variation explains a considerable proportion of the behavioural variation. In this case, we can further distinguish between individual and cultural plasticity because only cultural plasticity can produce geographic variation in behaviour in the absence of environmental differences. In sum, if we find for those behaviour patterns previously hypothesized to be cultural that their geographic variation is predicted by neither genetic nor ecological differences, whereas that in other behaviours is, we must accept a cultural interpretation for those behaviour patterns. Admittedly, the ability to distinguish between genetic and plasticity explanations comes at a price: cultural variants with a strong environmental imprint, and thus presumably the most adaptive ones, may go undetected. We therefore assume

that showing the presence of culture unrelated to environmental variation implies the presence of environmentally adaptive culture.

Separating individual from cultural plasticity is possible in principle through transplantation and social isolation experiments [32]. However, these experiments are often impossible for logistic, ethical and legal reasons, especially for primates, forcing us to resort to a parsimony approach by selecting the most consilient explanation for all relevant observations. Thus, in the case of great apes, the cultural interpretation of geographic variation in some behaviour patterns [15] is consistent with captive experiments showing reliable social transmission of novel skills [20] and observations suggesting selective visual attention for novel and especially difficult behavioural skills [18]. However, none of these studies directly addresses geographic variation as observed in the wild.

General methodological approach

Data on orang-utan behaviour were compiled from 11 study populations, with well over 100,000 hours of total observational effort [9] over 40 years. We included data on orang-utan behavioural ecology and social organisation and also considered behavioural variants that had previously been interpreted as cultural [10, 33], in two forms: (a) a set of 10 conspicuous and frequent behaviours without obvious environmental correlates, thus eliminating the role of possible observer bias, differential observation intensity, or environmental differences amongst sites, and (b) the total published set [33]. We also estimated genetic and environmental variation amongst these populations. We assessed the level of genetic dissimilarities between all populations using two mitochondrial DNA marker systems, which differ in their mutation rates, and therefore provide better dissimilarity estimates at shorter or longer periods since separation from a common ancestor. We quantified environmental differences between sites by constructing a data-matrix consisting of 10 variables to capture local dynamics in vegetation and climate. We used matrix permutation correlation tests [34] to investigate potential associations between the three behavioural dissimilarity matrices with genetic and environmental dissimilarity matrices. To estimate the proportions of the total variance in orang-utan behaviour attributable to either uniquely genetic or ecological differences between sites, we calculated squared partial matrix correlation coefficients, presented as unique variation explained**.** Details are given in the supplemental information.

Statistical analysis

All collated data on orang-utan biology (behaviour, genetics and ecology) at the various study sites were transformed into pairwise dissimilarity matrices. For each of the three behavioural as well as the environmental datasets, pairwise distances were expressed by Gower dissimilarity matrices, calculated in the 'ECODIST'-package [35] for R 2.10.1 [36]. The Gower dissimilarity metric [37] was chosen for its ability to deal with mixed variable types and its robustness against missing values [34, 38]. Genetic dissimilarity between populations was parameterised by φ_{ST} -values for mtDNA markers.

Matrix analyses were conducted using the Mantel permutation test implemented in the 'ECODIST'-package for R 2.10.1. Pearson correlation coefficients and associated bootstrapped 95% confidence intervals ($n_{bootstraps}$= 1,000) were calculated and assessed for statistical significance ($n_{permutations}$= 10,000). To estimate the unique proportions of the total variance in orang-utan behaviour attributable to either uniquely genetic or ecological differences between sites, we calculated squared partial matrix correlation coefficients, presented as unique variation explained independently by each of the two main variables, as suggested before [39]. This approach is valid only if collinearity between the two explanatory variables is sufficiently low. This condition was met, as the correlation between environmental dissimilarity and both genetic dissimilarity measures was low ($r_{Pearson}$ = 0.25 and 0.31 for HVR-I and mtDNA genes, respectively).

To account for the possibility that outliers may have exerted an undue influence on our analyses, we additionally calculated Spearman rank correlation coefficients and assessed these for statistical significance through Mantel matrix permutations.

Supplemental Information

Supplemental Information includes four tables and Supplemental Experimental Procedures, and can be found with this article online.

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References

1. Foster, S.A., and Endler, J.A. (1999). Geographic Variation in Behavior : Perspectives on Evolutionary Mechanisms, (New York: Oxford University Press).
2. Via, S., and Lande, R. (1985). Genotype-Environment interaction and the evolution of phenotypic plasticity. Evolution *39*, 505-522.
3. de Witt, T.J., Sih, A., and Wilson, D.S. (1998). Costs and limits of phenotypic plasticity. Trends Ecol Evol *13*, 1-8.
4. West-Eberhard, M.J. (2003). Developmental plasticity and evolution, (New York: Oxford University Press).
5. Whiten, A., and van Schaik, C.P. (2007). The evolution of animal 'cultures' and social intelligence. Phil Trans R Soc Lond B *362*, 603-620.
6. Galef, B.G., and Whiskin, E.E. (2003). Socially transmitted food preferences can be used to study long-term memory in rats. Learn Behav *31*, 160-164.
7. Laland, K.N., and Janik, V.M. (2006). The animal cultures debate. Trends Ecol Evol *21*, 542-547.
8. Tomasello, M. (1996). Do apes ape? In Social Learning in Animals: the Roots of Culture, C.M. Heyes and B.G. Galef, eds. (London: Academic Press), pp. 319-346.
9. Wich, S.A., Utami Atmoko S.S., Mitra Setia T, and van Schaik C.P., eds (2009). Orangutans : Geographic Variation in Behavioral Ecology and Conservation, (Oxford: Oxford University Press).
10. van Schaik, C.P., Ancrenaz, M., Borgen, G., Galdikas, B., Knott, C.D., Singleton, I., Suzuki, A., Utami, S.S., and Merrill, M. (2003). Orangutan cultures and the evolution of material culture. Science *299*, 102-105.
11. Wright, S. (1932). The roles of mutation, inbreeding, crossbreeding and selection in evolution. Proceedings of the 6th International Congress of Genetics, 356-366.
12. Slatkin, M. (1987). Gene flow and the geographic structure of natural population. Science *236*, 787-792.
13. Shettleworth, S.J. (2009). Cognition, Evolution and Behavior, (Oxford: Oxford University Press).
14. Heyes, C.M. (1994). Social learning in animals: categories and mechanisms. Biol Rev *69*, 207-231.
15. Richerson, P.J., and Boyd, R. (2005). Not By Genes Alone: How Culture Transformed Human Evolution, (Chicago, IL: University of Chicago Press).
16. Krützen, M., van Schaik, C., and Whiten, A. (2007). The animal cultures debate: response to Laland and Janik. Trends Ecol Evol *22*, 6.
17. Whiten, A., Goodall, J., McGrew, W.C., Nishida, T., Reynolds, V., Sugiyama, Y., Tutin, C.E.G., Wrangham, R.W., and Boesch, C. (1999). Culture in chimpanzees. Nature *399*, 682-685.
18. Jaeggi, A.V., Dunkel, L.P., van Noordwijk, M.A., Wich, S.A., Sura, A.A.L., and van Schaik, C.P. (2010). Social learning of diet and foraging skills by wild immature Bornean orangutans: Implications for culture. Am J Primatol *72*, 62-67.
19. Bastian, M.L., Zweifel, N., Vogel, E.R., Wich, S., and van Schaik, C.P. (2010). Diet traditions in wild orangutans. Am J Phys Anthrop *143*, 175-187.
20. Dindo, M., Stoinski, T., and Whiten, A. (2010). Observational learning in orangutan cultural transmission chains. Biol Letters *7*, 181-183.

21. Whiten, A. (2009). The identification of culture in chimpanzees and other animals: from natural history to diffusion experiments. In The Question of Animal Culture, K.N. Laland and B.G. Galef, eds. (Harvard: Harvard University Press).
22. Lynch, M., and Lande, R. (1993). Evolution and extinction in response to environmental change. In Biotic Interactions and Global Change, P. Kareiva, J. Kingsolver and R. Huey, eds. (Sunderland, MA: Sinauer).
23. Krützen, M., Mann, J., Heithaus, M.R., Connor, R.C., Bejder, L., and Sherwin, W.B. (2005). Cultural transmission of tool use in bottlenose dolphins (*Tursiops* sp.). Proc Natl Acad Sci *102*, 8939-8943.
24. Rendell, L., and Whitehead, H. (2001). Culture in whale and dolphins. Behav Brain Sci *24*, 309-382.
25. McComb, K., Moss, C., Durant, S., Sayialel, S., and Baker, L. (2001). Matriarchs as repositories of social knowledge. Science *292*, 491-494.
26. Perry, S., and Manson, J.H. (2003). Traditions in monkeys. Evol Anthrop *12*, 71-81.
27. Hunt, G.R., and Gray, R.D. (2003). Diversification and cumulative evolution in New Caledonian crow tool manufacture. P R Soc B *270*, 867-874.
28. Anholt, R.R.H., and Mackay, T.F.C. (2010). Principles of Behavioral Genetics, (Amsterdam, The Netherlands: Academic Press).
29. Langergraber, K., Boesch, C., Inoue, E., Inoue-Murayama, M., Mitani , J., Nishida, T., Pusey, A., Reynolds, V., Schubert, G., Wrangham, R., et al. (2010). Genetic and 'cultural' similarity in wild chimpanzees. P R Soc B *278*, 408-416.
30. Lande, R. (1976). The maintenance of genetic variability by mutation in a polygenic character with linked loci. Gen Res *26*, 221-235.
31. Latta, R.G. (1998). Differentiation of allelic frequencies at quantitative trait loci affecting locally adaptive traits. Am Nat *151*, 283-292.
32. Reader, S.M., and Biro, D. (2010). Experimental identification of social learning in wild animals. Learn Behav *38*, 265-283.
33. van Schaik, C.P. (2009). Geographic variation in the behavior of wild great apes: is it really cultural? In The Question of Animal Culture, K.N. Laland and B.G. Galef, eds. (Cambridge, MA: Harvard University Press), pp. 70-98.
34. Legendre, P., and Legendre, L. (1998). Numerical Ecology, Volume 20, 2nd Edition, (Amsterdam: Elsevier).
35. Goslee, S.C., and Urban, D.L. (2007). The ecodist package for dissimilarity-based analysis of ecological data. J Stat Softw *22*, 1-19.
36. R Development Core Team (2008). R: A language and environment for statistical computing. (Vienna, Austria: R Foundation for Statistical Computing).
37. Gower, J.C. (1971). A general coefficient of similarity and some of its properties. Biometrics *27*, 857-871.
38. Podani, J. (1999). Extending Gower's general coefficient of similarity to ordinal characters. Taxon *48*, 331-340.
39. Whitehead, H. (2009). How might we study culture? A perspective from the ocean. In The Question of Animal Culture, K.N. Laland and B.G. Galef, eds. (Cambridge, MA.: Harvard University Press), pp. 125-151.

Figure legends

Fig 1. Geographic locations of sites for which data on orang-utan biology were compiled. The different colours in the map correspond to estimated distributions of the currently recognized species and subspecies (Sumatra; dark red: *P. abelii*, Borneo; beige: *P. p. morio*, orange: *P. p. pygmaeus* and, dark orange: *P. p. wurmbii*). In addition, for each site details are provided on the type of information that was available (BE: Behavioural Ecology, SO: Social Organisation, PC: Putative Culture, ENV: Information on the local dynamics in vegetation and climate from remotely sensed and spatially interpolated sources. HVR-I, mtDNA genes: Number of individuals for which genetic data were obtained).

Fig 2. Uniquely genetic and environmental contributions to behavioural variation amongst orang-utans. Residual plots of genetic and ecological dissimilarity as a function of dissimilarities in behavioural ecology, social organisation and putative cultures, for two different genetic marker systems. Each dot represents a pairwise difference between sites. Blue dots denote comparisons within islands, red dots between islands.

Table 1. Genetic variation amongst orang-utan populations.

	HVR-I		mtDNA genes	
	Variance components	Percentage of variation	Variance components	Percentage of variation
Amongst populations	10.30	95.84%*	32.21	98.93%*
Within populations	0.65	4.16%	0.35	1.07%
Amongst islands	37.06	88.92%*	48.48	79.52%*
Amongst populations - within islands	3.90	9.36%*	7.81	12.81%*
Within populations	0.72	1.72%*	4.67	7.67%*

Variance components and percentage of variation explained for AMOVAs using HVR-I and mtDNA genes data for a flat structure and a partitioned dataset according to islands. Asterisks denote significance.

Table 2. Correlates of geographic variation in orang-utan behaviour.

		HVR-I				mtDNA genes			
x	**z**	**$r_{Pearson}$**	**95% CI**	**UVE**	**P_{Mantel}**	**$r_{Pearson}$**	**95% CI**	**UVE**	**P_{Mantel}**
		Δ Behavioural Ecology, 11 populations, 55 pairs							
φ_{ST}		0.368	0.248 - 0.489		0.002	0.361	0.171 - 0.512		0.002
Δ ENV		0.593	0.406 - 0.714		< 0.001	0.593	0.372 - 0.707		< 0.001
φ_{ST}	Δ ENV	0.199	0.116 - 0.310	**3.95%**	0.062	0.159	-0.045 - 0.311	**2.53%**	0.161
Δ ENV	φ_{ST}	0.529	0.309 - 0.631	**28.00%**	0.002	0.523	0.234 - 0.645	**27.36%**	0.003
		Δ Social Organisation, 7 populations, 21 pairs							
φ_{ST}		0.350	0.264 - 0.491		0.051	0.272	0.247 - 0.462		0.104
Δ ENV		0.544	0.369 - 0.650		0.022	0.544	0.369 - 0.668		0.022
φ_{ST}	Δ ENV	0.263	0.082 - 0.480	**6.90%**	0.113	0.237	0.139 - 0.444	**5.60%**	0.173
Δ ENV	φ_{ST}	0.504	0.252 - 0.651	**25.37%**	0.022	0.532	0.330 - 0.656	**28.30%**	0.022
		Δ Putative Culture (conspicuous & frequent elements), 9 populations, 36 pairs							
φ_{ST}		0.288	0.055 - 0.450		0.051	0.158	-0.311 - 0.403		0.174
Δ ENV		0.318	0.076 - 0.561		0.073	0.318	0.024 - 0.525		0.074
φ_{ST}	Δ ENV	0.223	0.042 - 0.344	**4.98%**	0.096	0.066	-0.297 - 0.273	**4.38%**	0.376
Δ ENV	φ_{ST}	0.262	0.037 - 0.475	**6.85%**	0.117	0.287	0.001 - 0.469	**8.22%**	0.116

Matrix Pearson correlation coefficients (Mantel and partial Mantel tests) for two different genetic marker systems of behavioural dissimilarity matrices with genetic (φ_{ST}; HVR-I and mtDNA genes) and environmental (Δ ENV) dissimilarities. The first two lines in each sub-table denote bivariate correlations, the next two to partial correlations (explanatory variables are indicated with x, variables that were partialled out with z). UVE = Unique proportions of variance explained. CI = Bootstrapped confidence intervals.

Figure 1
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	Site	Code	Species	Island	HVR-I	mtDNA genes	BE	SO	PC	ENV
1	Ketambe	KE	*Pongo abelii*	Sumatra	17	8	+	+	+	+
2	Suaq Balimbing	SB	*P. abelii*	Sumatra	15	12	+	+	+	+
3	Lower Kinabatangan	LK	*P. pygmaeus morio*	Borneo	8	6	+	-	+	+
4	Ulu Segama	US	*P. p. morio*	Borneo	-	-	+	-	-	+
5	Danum Valley	DV	*P. p. morio*	Borneo	16	13	+	-	-	+
6	Mentoko Kutai	KU	*P. p. morio*	Borneo	4	7	+	+	+	+
7	Gunung Palung	GP	*P. p. wurmbii*	Borneo	21	9	+	+	+	+
8	Tuanan	TU	*P. p. wurmbii*	Borneo	33	4	+	+	+	+
9	Sungai Lading	SL	*P. p. wurmbii*	Borneo	26	2	+	-	+	+
10	Sabangau	SA	*P. p. wurmbii*	Borneo	29	3	+	+	+	+
11	Tanjung Puting	TP	*P. p. wurmbii*	Borneo	3	-	+	+	+	+

Figure 2

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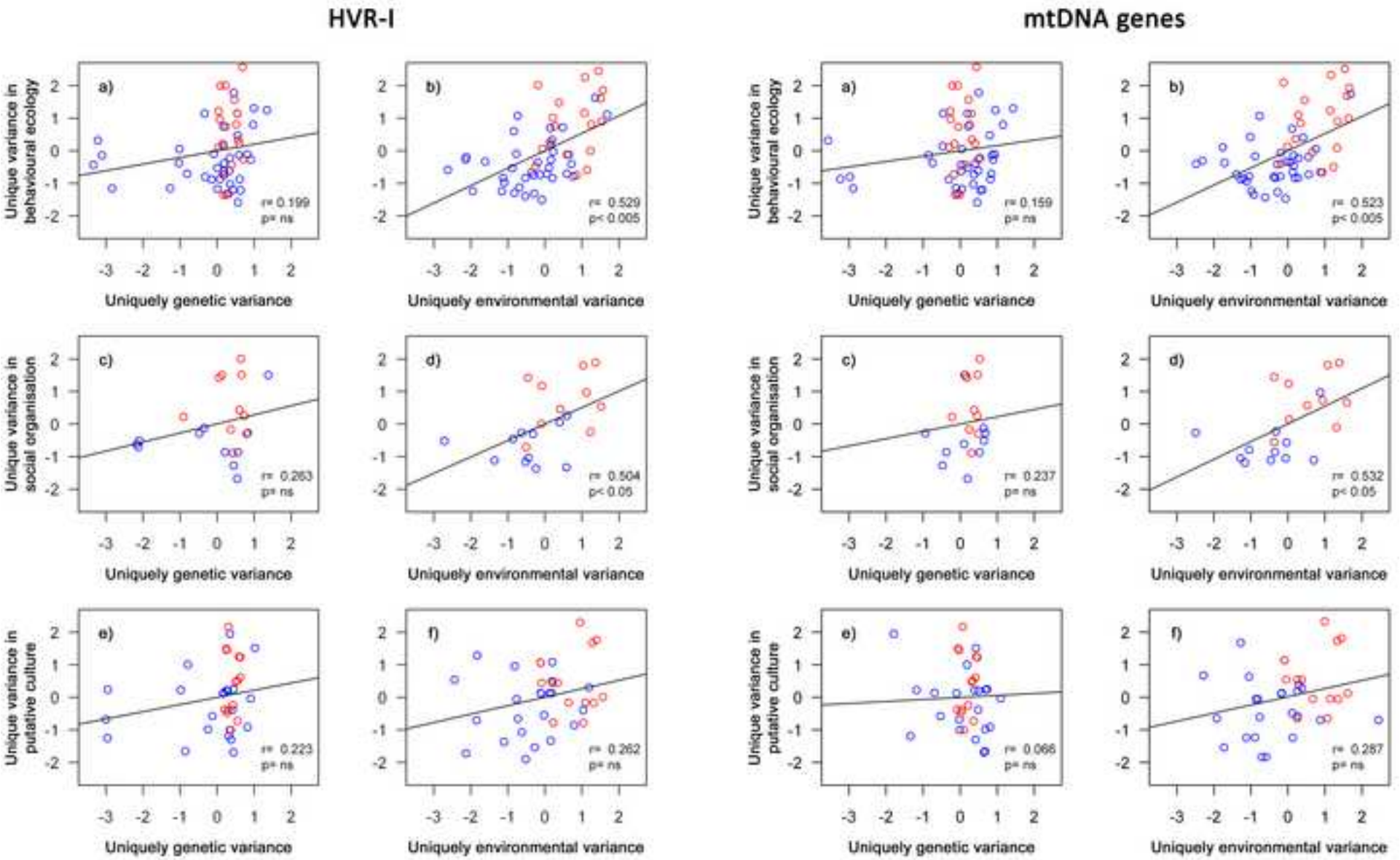

Supplemental Information

Culture and geographic variation in orang-utan behaviour

Michael Krützen, Erik P. Willems, and Carel P. van Schaik

Supplemental Inventory

1. Supplemental Tables

Table S1. Correlates of geographic variation in orang-utan putative cultures.

		HVR-I				mtDNA genes			
x	**z**	**$r_{Pearson}$**	**95% CI**	**UVE**	**P_{Mantel}**	**$r_{Pearson}$**	**95% CI**	**UVE**	**P_{Mantel}**
		Δ Putative Culture (all elements), 9 populations, 36 pairs							
φ_{ST}		0.114	-0.103 – 0.252		0.237	-0.067	-0.302 – 0.102		0.658
Δ ENV		0.343	0.071 – 0.514		0.081	0.343	0.071 – 0.514		0.081
φ_{ST}	Δ ENV	0.025	-0.084 – 0.152	**0.07%**	0.445	-0.194	-0.466 - -0.034	**3.77%**	0.838
Δ ENV	φ_{ST}	0.327	0.086 – 0.513	**10.68%**	0.090	0.384	0.190 – 0.559	**14.72%**	0.076

Pearson correlation coefficients of dissimilarity matrices of putative culture, using the full published set [1], on genetic (φ_{ST}; HVR-I and mtDNA genes) and environmental (Δ ENV) dissimilarities. The first two lines denote bivariate correlations, the next two to partial correlations (explanatory variables are indicated with x, variables that were partialled out with z). UVE = Unique proportions of variance explained. CI = Bootstrapped confidence intervals.

Table S2. Spearman correlations of geographic variation in orang-utan behaviour.

		HVR-I			mtDNA genes		
		$r_{Spearman}$	95% CI	P_{Mantel}	$r_{Spearman}$	95% CI	P_{Mantel}
x	**z**						
		Δ Behavioural Ecology, 11 populations, 55 pairs					
φ_{ST}		0.569	0.399 - 0.701	0.003	0.163	-0.040 - 0.347	0.201
Δ ENV		0.585	0.385 - 0.695	0.002	0.585	0.388 - 0.698	0.001
φ_{ST}	Δ ENV	0.297	0.200 - 0.404	0.027	0.059	-0.082 - 0.255	0.382
Δ ENV	φ_{ST}	0.336	0.212 - 0.481	0.016	0.571	0.404 - 0.685	0.002
		Δ Social Organisation, 7 populations, 21 pairs					
φ_{ST}		0.362	0.205 - 0.667	0.056	0.241	0.046 - 0.588	0.213
Δ ENV		0.568	0.446 - 0.712	0.033	0.568	0.446 - 0.661	0.033
φ_{ST}	Δ ENV	0.181	0.003 - 0.468	0.226	0.297	0.088 - 0.598	0.185
Δ ENV	φ_{ST}	0.495	0.149 - 0.658	0.036	0.586	0.406 -0.760	0.035
		Δ Putative Culture (conspicuous & frequent elements), 9 populations, 36 pairs					
φ_{ST}		0.313	0.034 - 0.471	0.070	-0.243	-0.443 - -0.042	0.863
Δ ENV		0.253	-0.032 - 0.444	0.129	0.253	-0.007 - 0.478	0.130
φ_{ST}	Δ ENV	0.202	0.011 - 0.340	0.128	-0.284	-0.430 - -0.081	0.897
Δ ENV	φ_{ST}	0.066	-0.089 - 0.237	0.367	0.292	0.072 - 0.478	0.104

Matrix Spearman correlation coefficients of two different genetic marker systems of behavioural dissimilarity matrices with genetic (φ_{ST}; HVR-I and mtDNA genes) and environmental (Δ ENV) dissimilarities. The first two lines in each sub-table denote bivariate correlations, the next two to partial correlations (explanatory variables are indicated with x, variables that were partialled out with z). CI = Bootstrapped confidence intervals.

Table S3. Contrasts in putative culture as a function of variation in social organisation.

			HVR-I				mtDNA genes			
x	**z_1**	**z_2**	**$r_{Pearson}$**	**95% CI**	**UVE**	***P*** **$_{Mantel}$**	**$r_{Pearson}$**	**95% CI**	**UVE**	***P*** **$_{Mantel}$**
			Δ Putative Culture (conspicuous & frequent elements), 9 populations, 36 pairs							
Δ SO			0.532	0.196 – 0.696		0.026	0532	0.196 – 0.696		0.026
Δ SO	φ_{ST}		0.491	0.143 – 0.667	**24.10%**	0.036	0.504	0.132 – 0.661	**25.37%**	0.030
Δ SO	Δ ENV		0.508	0.379 – 0.635	**25.82%**	0.022	0.508	0.379 – 0.635	**25.82%**	0.022
Δ SO	φ_{ST}	Δ ENV	0.481	0.253 – 0.583	**23.13%**	0.032	0.485	0.262 – 0.608	**23.50%**	0.028

Matrix Pearson correlation coefficients (Mantel and partial Mantel tests) of social organisation dissimilarities (Δ SO) with two different genetic (φ_{ST}; HVR-I and mtDNA genes) and environmental (Δ ENV) dissimilarities. The first line denotes the bivariate correlation, the remainder partial correlations (explanatory variables are indicated with x, variables that were partialled out with z). UVE = Unique proportions of variance explained. CI = Bootstrapped confidence intervals.

Table S4. Correlation between mitochondrial and microsatellite data.

HVR-I ~ mtDNA genes 55 pairs		HVR-I ~ Microsatellites 36 pairs		mtDNA genes ~ Microsatellites 36 pairs	
$r_{Pearson}$	P_{Mantel}	$r_{Pearson}$	P_{Mantel}	$r_{Pearson}$	P_{Mantel}
0.555	0.002	0.672	0.001	0.648	<0.001

Bivariate Pearson matrix correlation coefficients between φ_{ST} – values for HVR-I and mtDNA genes, and φ'_{ST} – values based on 12 microsatellite loci [2].

2. Supplemental Experimental Procedures

A. Generation of Behavioural Data

Data on behavioural ecology, social organisation, and behavioural elements that had previously been identified as cultural were largely collated from [3], and summarized in three broad categories. The first contained data on orang-utan behavioural ecology. Data were available from all 11 study sites and comprised the following 16 variables: the proportion of time spent (i) feeding, (ii) moving, (iii) resting, and (iv) the duration of average daily activity period for adult females. We further included values for diets of adult females, including the minimum and range of the proportions of fruit (v, vi) and invertebrates (vii, viii), as well as the maximum and range of the proportions of leaves (ix, x) and bark (xi, xii). Finally, data on adult female home range size (xiii), day journey length in the presence of dependent offspring (xiv), average number of nests that animals build during the day (xv), and whether or not males commonly travel on the ground (xvi), were added. Data not collated from [3] were taken from references [4-6] and http://130.54.114.7/meetings/2008/nettai2008/abstracts-e.html. Unpublished data were provided by N. Kuze (Kyoto University).

As a second category, a data-matrix capturing key parameters of the social organisation of orang-utans was constructed for 7 populations and consisted of 9 variables: (i) local density of adult females, (ii) adult female cluster size (females who show high home-range overlap and above-average association; estimated as average female home range size x local density [7], (iii) average party size in which adult females associate, (iv) average duration of adult female associations (party duration), (v) average duration of male long calls, (vi) average number of pulses per male long call, (vii) proportion of copulations by flanged males that are forced, (viii) proportion of copulations by unflanged males that are forced, and (ix) whether the local mating strategy of males is best described as ‘dominant males in distinct communities’ or ‘roving male promiscuity’ [8]. Data not collated from [3] were taken from references [5, 9-13]. Unpublished data were provided by C.P. van Schaik (University of Zurich) and M. Bastian (Philadelphia Zoo).

The third behavioural category contained behavioural elements that had previously been identified as cultural [1, 14]. Since observational effort on putatively cultural behaviours may have been biased across the 9 sites for which sufficiently detailed information was available, we analysed two different datasets within this category. The first dataset contained only highly

conspicuous and frequent behaviours (i.e. those behavioural elements in the animals' putatively cultural repertoire that, if present, would have been observed even in short-term studies not focusing on identifying unusual behaviour patterns). Moreover, this first set did not contain any behavioural elements whose varying presence could potentially be attributed to subtle environmental differences, however subtle, because the elements involved were ubiquitous in all orang-utan populations. This yielded a highly conservative list of 10 out of 24 distinct putatively cultural elements [1, 14] that were scored for their presence/absence: (i) c1: snag-riding, (ii) c2.4: kiss-squeak with leaves/leaf wipe, (iii) c11: raspberry, (iv) c14.29: leaf napkin/moss cleaning, (v) c15: branch as swatter, (vi) c17: tree-hole tool use, (vii) c24: slow loris eating, (viii) c25: nest-smack, (ix) c26: leaf-carrying, and (x) c34: *Asplenium* nest building. As a control, we also analysed all 24 previously identified potentially cultural elements [1].

B. Choice of Genetic Marker Systems

Recent estimates using data from the autosomal genome [15] and Y chromosome polymorphisms [16] showed a divergence time between Sumatran and Bornean orang-utans of about 400 kya and 168 kya, respectively. This recent divergence suggests that high-density microsatellite or single nucleotide polymorphism (SNP) analyses can be used to estimate genetic dissimilarities amongst populations. Because collecting such information requires high-quality DNA and a large number of individuals per population, this approach cannot be applied to wild great apes, for which only faeces or shed hair can be obtained non-invasively for a highly limited number of individuals. For the limited subset of sites for which we did have microsatellite information, we found a strong correlation between genetic differentiation estimates based on autosomal microsatellites and mitochondrial DNA (mtDNA) marker systems (Table S4). As in previous studies on explaining geographic variation in wild great ape populations [17, 18], we therefore used mtDNA markers to infer genetic distance between populations. This was because mtDNA data can be easily produced from faeces and hairs and could be collected for a sufficient number of individuals at most sites.

C. Generation of Genetic Data

We used both rapidly and slowly evolving mtDNA markers to calculate genetic dissimilarities between pairs of orang-utan populations. Matrix correlations were carried out independently for each marker set. The fast-evolving marker included 323 base pairs of the hyper-variable region I (HVR-I) of the mitochondrial DNA, which will best capture within-island population differentiation [2]. As the more slowly evolving marker, which performs better for larger between-island genetic divergence [16], we analysed 1,355 base pairs of three concatenated parts of coding mtDNA genes 16S rDNA, cytochrome b, and NADH-ubiquinone oxidoreductase chain 3 (referred to as 'mtDNA genes').

Faecal samples were extracted using a QIAamp DNA Stool Mini Kit on a QIAcube robotic workstation (both Qiagen) following the standard extraction protocol for human DNA extraction from stool samples with elution in 100 µl AE buffer. Blood samples were processed with a QIAamp DNA Blood Mini Kit (Qiagen) according to manufacturer's instructions and eluted in 100 µl AE buffer. Hair samples were extracted using an EZ1 DNA Investigator Kit on a BioRobot EZ1 Workstation (both Qiagen), applying the pre-treatment for DNA extraction from hair samples as described in the Investigator Kit manual with elution in 100 µl TE buffer. PCR conditions and sequencing reactions for HVR-I and the mtDNA genes were described previously [2, 16].

In order to avoid using haplotypes from single individuals multiple times in our analysis, we genotyped most populations using a panel of six highly polymorphic microsatellite markers [19, 20]. PCR amplifications were performed as multiplex reactions in an 8 µL volume containing 1 µL DNA, 4 µL Multiplex Master Mix (Qiagen), 0.8 µL primer mix, and 2.2 µL ddH_2O. Amplification conditions were: initial denaturation at 95°C for 15 minutes, followed by 40 cycles of 94°C for 30s, 58°C for 90s, 72°C for 1 min, and a final extension at 60°C for 30 mins. This was followed by capillary electrophoresis on the 3730xl DNA Analyzer (Applied Biosystems). Fragment-length polymorphisms were analysed using GENEMAPPER v4.0 (Applied Biosystems). Based on these data, we carried out an identity analyses as implemented in CERVUS v3.0 [21]. For the LK population, we used microsatellite data from a previous study [19]. If duplicate genotypes were found, only one mtDNA haplotype was retained in the data set. For three populations, we were not able to generate microsatellite data for all individual included in

the identity analysis. For GP, we used visual identification data collected during fieldwork. For KU and TP, we assumed all samples to be from different individuals.

Raw sequence data were edited in SEQUENCING ANALYSIS v5.2 (Applied Biosystems). Sequences were aligned with CLUSTALW [22] in BIOEDIT v7.0.9.0 [23], and collapsed using DAMBE v5.0.7.2 [24]. We calculated pairwise genetic distances (φ_{ST}) between populations for both marker systems using ARLEQUIN v3.5 [25]. For HVR-I and mtDNA genes, we applied the Tamura & Nei [26] distance with a gamma shape parameter of 0.344 and 0.281, respectively, as determined by JMODELTEST v0.1.1 [27]. For both marker systems, we also partitioned the genetic variance amongst populations using an AMOVA framework. Analyses were carried using the same parameters as for pairwise genetic measures in ARLEQUIN, v3.5. Significance of variance was assessed by 1,000 random permutations. For the one population in our sample from which no genetic data were available (US), we inferred pairwise φ_{ST} values by taking the inverse geographic distance weighted average computed from DV and LK, the two nearest sites.

D. Generation of Environmental Data

Prevalent environmental conditions were assessed across sites by utilizing a Geographic Information System ARCGIS [28]. For each site, vegetational dynamics were characterized by data on primary productivity and potential evapotranspiration. Information on primary productivity was obtained from a well-established remotely sensed spectral correlate of terrestrial photosynthetic activity, the Normalized Difference Vegetation Index, NVDI [29, 30]. Data used in this study were obtained from the Advanced Very High Resolution Radiometer [31], processed by the GIMMS-group at NASA's Goddard Space Flight Centre [32]. Monthly maximum value composites [33] were constructed and used to calculate: (i) average annual maximum NDVI, and (ii) annual seasonality, expressed by the coefficient of variation [CV] in averaged monthly values. In parallel, (iii) annual total Potential EvapoTranspiration (PET), and (iv) annual seasonality ($CV_{monthly\ totals}$) were calculated from the CGIAR-CSI Global-PET Geospatial Database [34].

Climatic information was obtained from spatially interpolated datasets [35] and provided information on (v) mean annual temperature and (vi) the averaged diurnal range therein. In the tropics, diurnal variation in temperature typically exceeds seasonal variation and is therefore considered to be more informative to the study of primate behaviour [36]. Other climatic

information from this source was (vii) total annual precipitation and (viii) annual seasonality therein ($CV_{monthly\ totals}$). Lastly, we used data on (ix) annual mean cloud cover and (x) seasonality therein ($CV_{monthly\ mean}$) [37]. The inclusion of cloud cover as a climatic variable of relevance to orang-utan behaviour was based on a recent study which showed that over both Sumatra and Borneo incipient solar radiation, which is negatively related to cloud cover, is the single most limiting climatic condition to habitat productivity [38].

3. Supplemental References

1. van Schaik, C.P., Ancrenaz, M., Djojoasmoro, R., Knott, C.D., Morrogh-Bernard, H.C., Nuzuar, Odom, K., Utami Atmoko, S.S., and van Noordwijk, M.A. (2009). Orangutan cultures revisited. In Orangutans: Geographic variation in behavioral ecology and conservation, S. Wich, S.S. Utami Atmoko, T. Mitra Setia and C.P. van Schaik, eds. (New York: Oxford University Press Inc.), pp. 299-310.
2. Arora, N., Nater, A., van Schaik, C., Willems, E.P., van Noordwijk, M.A., Goossens, B., Morf, N., Bastian, M.L., Knott, C.D., Morrogh-Bernard, H.C., et al. (2010). Effects of Pleistocene glaciations and rivers on the population structure of Bornean orangutans (*Pongo pygmaeus*). Proc Natl Acad Sci *107*, 21376-21381.
3. Wich, S.A., Utami Atmoko, S.S., Mitra Setia, T., and van Schaik, C.P. (2009). Orangutans: Geographic Variation in Behavioral Ecology and Conservation, (Oxford: Oxford University Press).
4. Fox, E.A., van Schaik, C.P., Sitompul, A., and Wright, D.N. (2004). Intra-and interpopulational differences in orangutan (*Pongo pygmaeus*) activity and diet: implications for the invention of tool use. Am J Phys Anthrop *125*, 162-174.
5. Bastian, M.L. (2008). Effects of a riverine dispersal barrier on cultural similarity in wild bornean orangutans (*Pongo pygmaeus wurmbii*). Ph.D. thesis (Durham, NC: Duke University).
6. Zweifel, N. (2008). Dietary differences between two orangutan populations in Central Kalimantan, Indonesia: indications for individual and social learning. M.Sc. thesis (Zurich: University of Zurich).
7. Singleton, I., Knott, C.D., Morrogh-Bernard, H.C., Wich, S.A., and van Schaik, C.P. (2009). Ranging behavior orangutan females and social organization. In Orangutans : geographic variation in behavioral ecology and conservation, S.A. Wich, S.S. Utami Atmoko, T. Mitra Setia and C.P. van Schaik, eds. (Oxford: Oxford University Press).
8. Utami Atmoko, S.S., Mitra Setia, T., Goossens, B., James, S.S., Knott, C.D., Morrogh-Bernard, H.C., van Schaik, C.P., and van Noordwijk, M.A. (2009). Orangutan mating behavior and strategies. In Orangutans : geographic variation in behavioral ecology and conservation, S.A. Wich, S.S. Utami Atmoko, T. Mitra Setia and C.P. van Schaik, eds. (Oxford: Oxford University Press).
9. Galdikas, B. (1988). Orangutan diet, range, and activity at Tanjung Puting, Central Borneo. Int J Primatol *9*, 1-35.
10. Mitani, J.C., Grether, G.F., Rodman, P.S., and Priatna, D. (1991). Association among wild orang-utans: sociality, passive aggregations or chance? Anim Behav *42*, 33-46.

11. Rodman, P.S. (1977). Feeding behaviour of orang-utans of the Kutai Nature Reserve, East Kalimantan. (London: Academic Press), pp. 383-413.
12. Morrogh-Bernard, H.C. (2009). Orang-utan behavioural ecology in the Sabangau peat-swamp forest, Borneo. Ph.D. thesis (Cambridge, UK: University of Cambridge).
13. Delgado, R.A. (2003). The function of adult male long calls in wild orangutans (*Pongo pygmaeus*). Ph.D. thesis (Durham, NC: Duke University).
14. van Schaik, C.P., Ancrenaz, M., Borgen, G., Galdikas, B., Knott, C.D., Singleton, I., Suzuki, A., Utami, S.S., and Merrill, M. (2003). Orangutan cultures and the evolution of material culture. Science *299*, 102-105.
15. Locke, D.P., Hillier, L.W., Warren, W.C., Worley, K.C., Nazareth, L.V., Muzny, D.M., Yang, S.P., Wang, Z.Y., Chinwalla, A.T., Minx, P., et al. (2011). Comparative and demographic analysis of orang-utan genomes. Nature *469*, 529-533.
16. Nater, A., Nietlisbach, P., Arora, N., van Schaik, C., Singleton, I., Goossens, B., Knott, C.D., Morrogh-Bernard, H.C., Warren, K.S., Verschoor, E.J., et al. (2011). Phylogeography and population history of orangutans (*Pongo* spp.) based on mtDNA and Y-chromosomal data. Mol Biol Evol *28*, 2275-2288.
17. Langergraber, K., Boesch, C., Inoue, E., Inoue-Murayama, M., Mitani , J., Nishida, T., Pusey, A., Reynolds, V., Schubert, G., Wrangham, R., et al. (2011). Genetic and 'cultural' similarity in wild chimpanzees. P R Soc B *278*, 408-416.
18. Lycett, S.J., Collard, M., and McGrew, W.C. (2007). Phylogenetic analyses of behavior support existence of culture among wild chimpanzees. Proc Natl Acad Sci *104*, 17588-17592.
19. Goossens, B., Chikhi, L., Jalil, M.F., Ancrenaz, M., Lackman-Ancrenaz, I., Mohamed, M., Andau, P., and Bruford, M.W. (2005). Patterns of genetic diversity and migration in increasingly fragmented and declining orang-utan (*Pongo pygmaeus*) populations from Sabah, Malaysia. Mol Ecol *14*, 441-456.
20. Nietlisbach, P., Greminger, M., Nater, A., Arora, N., and Krützen, M. (2010). A multiplex-system to target 16 male-specific and 15 autosomal genetic markers for orang-utans (genus: *Pongo*). Cons Gen Res *2*, 153-158.
21. Marshall, T.C., Slate, J., Kruuk, L.E.B., and Pemberton, J.M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol *7*, 639-655.
22. Thompson, J., Higgins, D., and Gibson, T. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl Acid Res *22*, 4673-4680.
23. Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment program for Windows 95/98/NT. Nucl Acid Symp Ser *41*, 95-98
24. Xia, X., and Xie, Z. (2001). DAMBE: Data analysis in molecular biology and evolution. J Hered *92*, 371-373.
25. Excoffier, L., and Lischer, H.E.L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Res *10*, 564-567.
26. Tamura, K., and Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol *10*, 512-526.
27. Posada, D., and Crandall, K.A. (1998). MODELTEST: testing the model of DNA substitution. Bioinformatics *14*, 817-818.

28. ESRI (2008). ESRI Data & Maps 9.3. (Redlands, CA USA: Environmental Systems Research Institute, Inc).
29. Kerr, J.T., and Ostrovsky, M. (2003). From space to species: ecological applications for remote sensing. Trends Ecol Evol *18*, 299-305.
30. Pettorelli, N., Vik, J.O., Mysterud, A., Gaillard, J.M., Tucker, C.J., and Stenseth, N.C. (2005). Using the satellite-derived NDVI to assess ecological responses to environmental change. Trends Ecol Evol *20*, 503-510.
31. Cracknell, A.P. (2001). The exciting and totally unanticipated success of the AVHRR in applications for which it was never intended. Adv Space Res *28*, 233-240.
32. Tucker, C.J., Pinzon, J.E., Brown, M.E., Slayback, D.A., Pak, E.W., Mahoney, R., Vermote, E.F., and El Saleous, N. (2005). An extended AVHRR 8-km NDVI dataset compatible with MODIS and SPOT vegetation NDVI data. Int J Remote Sens *26*, 4485-4498.
33. Holben, B.N. (1986). Characteristics of maximum-value composite images from temporal AVHRR data. Int J Remote Sens *7*, 1417-1434.
34. Trabucco, A., and Zomer, R.J. (2009). Global Potential Evapo-Transpiration (Global-PET) Geospatial Database. CGIAR Consortium for Spatial Information, Available from the CGIAR-CSI GeoPortal at: http://www.csi.cgiar.org/.
35. Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., and Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. Int J Climatol *25*, 1965-1978.
36. van Schaik, C.P., Madden, R., and Ganzhorn, J.U. (2005). Seasonality and primate communities. In Seasonality in Primates, D.K. Brockmann and C.P. van Schaik, eds. (Cambridge: Cambridge University Press), pp. 445-463.
37. Mitchell, T.D., and Jones, P.D. (2005). An improved method of constructing a database of monthly climate observations and associated high-resolution grids. Int J Climatol *25*, 693-712.
38. Boisvenue, C., and Running, S.W. (2006). Impacts of climate change on natural forest productivity: evidence since the middle of the 20th century. Global Change Biol *12*, 862-882.